PROJECT NUMBER: 1730
PROJECT TITLE: Plant Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
PERIOD COVERED: July, 1987

I. TOBACCO-IDENTICAL PRESERVATIVES

A. Objective: To develop procedures and to establish microbiological screens for the evaluation of new, tobacco-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. Status:

1. Research Proposal: A research proposal has been submitted to management outlining the methods and approaches that will be used to screen natural tobacco materials for preservative (antimicrobial) activity (1). The principal targets include:

   a. Readily available, known biochemicals that have been approved for use as preservatives on tobacco either in the US or Europe (such as those appearing on the GRAS, Hunter or other lists),

   b. Various chemical fractions that have been isolated from tobacco and that are currently available at PM R&D for further testing (such as duvatriene-diols, sucrose esters, etc., see reference 2),

   c. Aqueous and organic extracts of oriental tobaccos (see reference 1).

   While some revisions are currently being made to this proposal, some of the preliminary experimental work has been initiated (see below).

2. Microbiological Screens: Several experiments have recently been conducted in a Nutrient Broth system containing 5% basal salts and MES buffer (pH 5.7) to test the potassium propylparaben screening procedures developed by Ola Mallory and Walter Hempfling. A pure culture of Bacillus coagulans isolated from chemically-altered ('spoiled') SEL served as the initial test organism and was grown in the presence of several concentrations of K-propylparaben (including 0, 100, 150 and 300 ppm). The growth of triplicate control and test cultures was monitored at 45 minute intervals by following the change in optical density at 650 nm over a period of 180 minutes (some cultures were followed overnight in order to obtain additional, end-point data).

3. General Microbiology: Procedures are being developed to store pure microbial cultures isolated from SEL as spores. This would replace the current practice of perpetuting such isolates over extended periods of time (months) via the

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intermittent passage and refrigerated storage of vegetative cells.

C. **Results**: Repeatable results were obtained in each of five successive screening experiments corroborating the results reported earlier by Hempfling and Mallory (3). In each case, the 150 ppm level of K-pp produced about half-maximal growth while the 300 ppm level inhibited growth completely under the experimental conditions employed.

D. **Conclusions**: None to be reported at this time.

E. **Plans**:

1. Test Materials: Discussions are currently being held with a variety of R & D personnel to help establish a list of prospective preservatives for testing. These materials would be selected from chemical fractions that have already been isolated from tobacco at PM R&D as well as from biochemicals which appear on published lists of known preservatives that have been accepted for use on tobacco either within the USA or internationally.

2. Microbiological Screens: Additional test screens are planned for August in which 'sham' unknown preservatives (such as various antimicrobial agents, etc.) will be evaluated in blind tests against the propylparaben/B. coagulans control screen previously described.

F. **References**:

1. Uydess, I. L. and Teng, D. M., "Development of microbiological screens for identifying and evaluating tobacco-identical preservatives as replacements for propylparaben", PM Memo to Dr. E. B. Sanders, in press.

2. Personal communications, Dr. Richard Izac, Dr. Jeff Chan, and Dr. Rett Southwick.

3. Personal communications, Miss Ola Mallory and Dr. Walter Hempfling.

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